Flow Cytometry Core, MRC 3030
Moffitt Cancer Center
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TITLE: Quick guide for the start-up, maintenance, acquisition, and shutdown of the xMAP IntelliFlex DR-SE (Dual Reporter-Side Eject).

EFFECTIVE DATE: 25th August, 2025

I. PURPOSE

This quick guide details the procedure for starting up, quality control, acquiring, and shutting down the xMAP IntelliFlex DR-SE (Luminex) multiplexing platform.

II. REFERENCES

1. https://www.thermofisher.com/us/en/home/life-science/antibodies/immunoassays/procartaplex-assays-luminex/luminex-instruments/luminex-xmap-intelliflex.html
2. https://www.youtube.com/watch?v=nG9HoKWLmHo
3. https://us.diasorin.com/sites/default/files/products-documentation-tool/89-00002-00-826.pdf

III. MATERIALS AND REAGENTS

DiH2O: Deionized water.

• 70% alcohol (isopropanol or ethanol).
• 10% Pure Bright Bleach.
• DiH2O.
• IntelliFlex reservoir block.
• xMAP IntelliFlex Calibration Kit **(Cat#IFXCALK20, ThermoFisher Scientific**).
• xMAP IntelliFlex Performance Verification Kit **(Cat#IFXPVER20, ThermoFisher Scientific**).
• xMAP Sheath Fluid Plus **(Cat#4050021, ThermoFisher Scientific**).

A. START-UP

1. Check the waste and sheath tanks below the IntelliFlex. Empty the waste if necessary. Fill approximately 1/10 of the waste tank with new bleach. Replace the sheath tank if required.
2. Push and hold the soft power button on the right side of the instrument for 2 to 3 seconds.
3. Tap the screen to pull up the system sign-in page.

   a. Enter the username and password credentials for the instrument.
4. The screen will open to the IntelliFlex software page, where the upper left software navigation menu and dashboard page are now accessible.
5. Laser warmup must occur before any data acquisition. Lasers will automatically start warming up when the machine is turned on, and it takes 30 minutes to fully complete this warm-up period.

B. **QUALITY CONTROL**

1. Reservoir and off-plate strip well probe height calibration:
   a. Select the upper left software navigation menu button.
   b. Select “maintenance”.
   c. Select “probe height”.
   d. Select the eject button in the lower right corner of the screen to eject the plate carrier.
   e. Place the reservoir block on the plate carrier, with one off-plate strip well in the middle row. Verify that no liquid is present in the strip well or reservoirs of the block.
   f. Select the “eject’ button to retract the plate carrier again.
   g. Press the “auto calibrate” in the reservoirs section.
   h. Press the “auto calibrate” in the off-plate strip section.
2. New hard plate probe calibration:
   a. Select the upper left software navigation menu button.
   b. Select maintenance.
   c. Select probe height.
   d. Place a plate of the desired type for probe adjustment on the plate carrier, oriented so that A1 is in the top left corner. Confirm that no liquid is present in the plate.
   e. Select the “eject” button to retract the plate carrier again.
   f. Select “New” to define a new plate type.
   g. Select “96” or “384” -well plate option depending on the plate type used.
   h. Press “Auto calibrate” in the Plate section.
   i. Save new probe height setting if desired.
3. Daily fluidics startup:
   a. Select the upper left software navigation menu button.
   b. Select “Daily Start-up” from the maintenance and cleaning command list.
   c. Fill the IntelliFlex reservoir block with 70% isopropanol or 70% ethanol and with DIH2O according to the plate layout diagram outlined on the screen.
   d. Select the “eject” button to eject the plate carrier. Load the IntelliFlex reservoir block. Select “eject” again to retract the plate carrier.

e. Select the “play” button on the lower right corner of the screen. Status updates will be visible on the screen until the routine is completed.

4. Running calibration and performance verification:
   a. Select the upper left software navigation menu button.
   b. Select “maintenance”.
   c. Select “Cal/Ver”
   d. Locate the calibration and performance verification kits. The bottles in the kit will have associated lot IDs listed on them, which will be available for selection on the drop-down menus of the IntelliFlex once the respective kits have been imported into the system.
   e. Under the “Calibration”, “Performance Verification”, and “Fluidics Verification” dropdown menus, select the correct lot IDs for the kits currently in use. As lot IDs are selected, the run boxes become checked and the reservoir block/off-plate strip well diagram on the screen will indicate where to add each of the reagents in a color-coded fashion. Additionally, the diagram will indicate where to fill the reservoir block with DIH2O and 70% isopropanol or 70% ethanol.
   f. On the reservoir block, place a single off-plate strip well on the top row of the block.
   g. Vigorously hand-shake/invert the calibration, verification, and fluidics bottles and add six drops per indicated well of the necessary reagent according to the diagram outlined on the screen.
   h. Select the “eject” button to eject the plate carrier. Load the IntelliFlex reservoir block/off-plate strip well containing added beads, DIH2O, and alcohol washing reagents. Select “eject’ again to retract the plate carrier. Then select the “play” button to run.
   i. Once completed, verify that all calibrations and verifications have passed and are sufficient for machine usage. Once verified, the system will be ready for data acquisition.

C. DATA ACQUISITION

1. Click the upper left software navigation menu button. Select “Plate configuration.” Select “Layout.”
2. Select “New” to create a new layout or use the dropdown menu to load a previously created layout.
3. Select “96-well” or “384-well” plate by toggling to the 96-well or 384-well icons on the lower left corner of the screen.

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4. Define the well locations for the samples listed in the sample plate by highlighting the designated wells and then selecting the appropriate sample type.
5. Once the layout is completed, select “Save as” to save the layout for later use.
6. Click the upper left software navigation menu button. Select “Plate configuration.” Select “Panel.”
7. Select the bead regions to include by selecting the required analyte number on the screen.
    a. If necessary, update the minimum bead count for each region under the “min beads” column.
    b. If desired, update the name of each analyte within the analyte list. This is not required for acquisition.
8. Select “Save as” to save the layout for later use.
9. Select the upper left software navigation menu button. Select “Plate Configuration”. Select “Acquisition”.
10. Under acquisition, choose the correct assay settings for plate type, read direction, operating mode, PMT mode, uptake volume, timeout, bead type, doublet discrimination minimum, doublet discrimination maximum, and heater settings according to the multiplexing assay being run.
    a. At the bottom of the page, under maintenance modes, you can select a pre-assay cleaning routine, a clean routine during the assay run, or a post-assay cleaning routine.
    b. You can start designing a new list of these settings by selecting “Save as” at the top right of the page at the end of the design process or selecting from a previously created list of settings in the dropdown menu under “settings” at the top of the page.
11. Once each of the assay configuration settings is loaded into the “Layout,” “Panel,” and “Acquisition” tabs, select the “eject” button to eject the plate carrier and load the desired plate along with any necessary washing reagents if the “maintenance” section of the acquisition tab was chosen. Select the “eject” button again to retract the plate into the machine.
12. Verify that the chosen assay settings are correct by looking in the plate overview window. When confirmed, select the “Run plate” button in the overview window.
    a. After each independent parameter has been selected, the three independent parameters (layout, panel, and acquisition) can be saved together as a protocol for repeat use by going to the upper left software navigation menu button, selecting “plate configuration,” and selecting “Save” at the very top of the screen in the blue ribbon. Saving a protocol is not required for data acquisition.
13. After all designated wells on the plate have been run, a message stating that the run has been completed will be displayed.

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14. To export results, navigate to the history tab by selecting the upper left software navigation button and selecting “History.”
15. In the calendar on the left portion of the screen, select the acquisition date of the plate file you want to export. Selecting the correct date will display a list of acquired data under the “Plates” tab on the left side of the screen. Select the check box next to the desired plate/acquisition in the list.
16. Select “xPONENT” under “Report Type” in the lower left portion of the window. This will automatically default the file type to .csv for future xPONENT program use.
17. Select “Export” on the bottom right of the window.

D. SHUTDOWN
1. Select the upper left software navigation menu button. Select “maintenance”. Select “Routines”.
2. Select “Daily shutdown” from the cleaning commands listed.
3. Fill the IntelliFlex reservoir block with 10% Pure Bright Bleach and DIH2O according to the plate layout diagram outlined on the screen.
4. Select the “eject” button to eject the plate carrier and load the IntelliFlex reservoir block. Select “eject” again to retract the plate carrier.
5. Select the “play” button on the lower right corner of the screen.
6. Once completed, remove the IntelliFlex reservoir block. Rinse the block with DIH2O and allow the block to dry.
7. Regardless of whether the screen is loaded, push the soft power button on the instrument's right side. Do not press and hold down the soft power button but wait until a window appears to confirm the shutdown process. Select “Yes” to confirm shutdown.
8. Empty the waste and refill with 1/10 of bleach if necessary.
9. Replace the sheath tank, if necessary.

E. TROUBLESHOOTING
1. For troubleshooting issues, follow the xMAP INTELLIFLEX® 2.1 System Guide, **Pages 105-116**.
2. If any other major problem occurs, contact Luminex Technical Support at 1-877-785-2323.
Contact outside the U.S. and Canada: +1 512-381-4397 International: + 800-2939-4959
Fax: 512-219-5114 Email: support@luminexcorp.com.

V. REVISION HISTORY
27th August, 2025

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